ORIGINAL ARTICLE

Size bias of fragile X premutation alleles in late-onset movement disorders

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Background: Fragile X-associated tremor/ataxia syndrome (FXTAS), caused by premutation expansions (55–200 CGG repeats) of the *FMR1* gene, shares clinical features with other movement disorders, particularly in the domains of gait ataxia, intention tremor and parkinsonism. However, the prevalence of FXTAS within other diagnostic categories is not well defined.

Methods: A meta-analysis was conducted of all published (n = 14) genetic screens for expanded *FMR1* alleles to assess the prevalence and CGG-repeat size bias of *FMR1* premutation alleles in those populations.

Results: In men with late-onset cerebellar ataxia, the prevalence of premutation alleles (1.5%; 16/1049) was 13 times greater than expected based on its prevalence in the general population (2%; 16/818 for age of onset >50 years; odds ratio 12.4; 95% confidence interval 1.6 to 93.5). Meta-analysis of CGG-repeat data for screened patients with premutation alleles shows a shift to larger repeat size than in the general population (p<0.001). 86% (19/22) of premutation alleles were larger than 70 repeats in the patients screened, whereas only approximately 22% of premutation alleles are larger than 70 repeats in the general population.

Conclusions: Expanded *FMR1* alleles contribute to cases of late-onset sporadic cerebellar ataxia, suggesting that *FMR1* genetic testing should be carried out in such cases. The biased distribution of *FMR1* allele sizes has substantial implications for genetic counselling of carriers with smaller alleles who are at a low risk of developing FXTAS, and suggests that the estimated prevalence of FXTAS among men >50 years of age in the general population may be two to threefold lower than the initial figure of 1 in 3000.

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a recently identified neurodegenerative disorder that seems to be restricted to carriers of premutation alleles (55–200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene.[1–3] The syndrome primarily affects adult men >50 years, although some female carriers also develop the clinical features of FXTAS.[4–6] The core clinical features of FXTAS are progressive gait ataxia and intention tremor, with associated clinical features, including peripheral neuropathy, decline of memory and cognition, and autonomic dysfunction.[3 7] FXTAS is completely distinct from the neurodevelopmental disorder, fragile X syndrome, which generally affects children with *FMR1* alleles containing >200 CGG repeats (full mutation).

Neuroradiological features of FXTAS include prominent white matter disease in the periventricular, subcortical and middle cerebellar peduncle (MCP) regions on T2-weighted or fluid-attenuated inversion recovery magnetic resonance imaging.[8] Increased signal intensities of the MCPs are a common feature of FXTAS, occurring in approximately 60% of cases.[3 7 8] Radiological features of FXTAS also include global brain atrophy, most evident in the frontal and parietal regions, as well as in the pons and cerebellum, with the degree of brain atrophy associated with both the presence and severity of the tremor and ataxia and CGG repeat size.[8 9]

The principal neuropathological characteristic of FXTAS is an eosinophilic, ubiquitin-positive inclusion that is located in the nuclei of neurones and astrocytes in broad distribution throughout the brain and spinal column.[10] The inclusions are τ-protein and α-synuclein negative; however, they do contain *FMR1* mRNA,[11] which is now thought to exert a direct toxic gain of function, leading to the tremor/ataxia disorder.[12]

A recent study of the penetrance of tremor and ataxia among adult carriers of premutation (*FMR1*) alleles, ascertained through families with known fragile X syndrome probands, showed that more than one third of male carriers >50 years of age had both tremor and ataxia.[2] Moreover, the penetrance increased with age, exceeding 50% for men in their 70s and 80s. From these penetrance figures and the prevalence of premutation alleles (approximately 1/800 males; 1/250 females) in the general population,[13 14] it is estimated that as many as 1 in 3000 men >50 years in the general population will develop FXTAS. This estimate, however, is predicated on the assumption that the penetrance and severity of the neurological disorder is not a strong function of the number of CGG repeats; that is, the penetrance among carriers of small premutation alleles (about 55–70 CGG repeats) should be roughly equal to the penetrance among carriers with larger premutation alleles.

Three observations suggest that the true prevalence may be lower than the above estimate. Firstly, the preponderance of alleles in the family-based studies are in the 70–100 CGG-repeat range, reflecting the greater propensity for the transmission of full mutation alleles from larger premutation alleles[15]; thus, carrier grandfathers of fragile X syndrome children would have a biased (larger) distribution of premutation alleles relative to the size distribution in the general population.[13 14] Secondly, the screening of adult populations with movement disorders, which should not reflect any transmission bias, suggests that the distribution of

Abbreviations: FXTAS, fragile X-associated tremor/ataxia syndrome; MCP, middle cerebellar peduncle; MRI, magnetic resonance imaging; MSA, multiple system atrophy alleles may be shifted to a larger repeat size than in the general population. Thirdly, preliminary data indicate that the severity of both neuropathological and radiological features of FXTAS are a function of the CGG repeat size,[9 10] suggesting that carriers of smaller repeats may be less likely to present clinically.

Taken together, the above observations suggest that there may be a CGG-repeat size bias for clinical involvement in FXTAS, with those with the smallest repeat expansions having a lower likelihood of clinical involvement or of having only a mild phenotype. Such a size bias, if present, would have substantial implications for genetic counselling and for our expectations regarding the prevalence of premutation alleles within the major movement disorder populations. To begin to deal with this issue, the following meta-analysis compares the clinical and molecular data from the population-based studies with those of the cases identified through fragile X families. This study should help to refine our estimate for the prevalence of FXTAS cases in the general population, and shed light on the related question of allele size effects, including a possible threshold for clinical involvement in FXTAS.

METHODS

Data from premutation allele screening in movement disorder and family-based populations

All studies reported in this meta-analysis were carried out in accordance with their respective institutional review board-approved protocols.

Clinical and molecular data were analysed from 14 articles reporting results for *FMR1* testing on adults referred to movement disorder clinics.[6 16–28] All of the aforementioned studies were retrospective: the patients did not have a reported family history of fragile X syndrome. Diagnostic categories included multiple system atrophy (MSA), Parkinson's disease, essential tremor and cerebellar ataxia, as well as diagnostic subcategories of the main disorders. The population samples were for the most part clearly defined in the screening studies. Throughout this article, the size of a premutation allele is defined as ranging between 55 and 200 repeats.

Among the 14 screening studies, four screened a total of 663 cases of MSA.[16 19 22 26] The screened populations included 280 cases of MSA-C (cerebellar), 341 cases of MSA-P (parkinsonism) and 42 cases of possible MSA that could not be classified as either cerebellar or parkinsonian. The MSA diagnosis was defined according to the Gilman criteria,[29] with documented autonomic dysfunction and without cognitive decline. The Parkinson's disease group included 605 idiopathic and 62 atypical cases.[22 23 27] The essential tremor groups ($n = 348$)[17 22 27] were defined by the following criteria: action or intention tremor, and absence of bradykinesia, rigidity, or gait or postural changes (consensus statement of the Movement Disorder Society[30]). The 73 patients screened by Garcia Arocena *et al*[17] had familial essential tremor as defined by the presence of one or more first-degree relatives with essential tremor. The cerebellar ataxia group (1598 patients) was defined by a previous referral for spinocerebellar ataxia genetic testing.[6 16 18 20–22 24 25 28] Routine genetic testing is not available for patients presenting with sporadic Parkinson's disease, MSA or essential tremor. Therefore, these groups of patients had previously been defined on the basis of strict clinical criteria.

Of the 3265 patients screened, 935 were women (MSA 297 cases; ataxia 549 cases; essential tremor 78 cases; and atypical Parkinson's disease 11 cases). Information on age of onset or age at evaluation was provided by all studies except Macpherson *et al*[20] and Kamm *et al*.[26] The overall mean (SD) age of onset in the cases screened was 53.4 (5.8) years (based on data available in 7/14 studies, range: 42–67 years[16–18 21–23 27]). Four studies reported age at clinical evaluation.[19 24 25 28]

For patients with FXTAS identified through family studies, clinical and molecular data were summarised from studies previously published by the Hagerman collaborative group.[1–3 7 8]

Comparison of premutation allele distributions in the general population and in FXTAS patient groups

We carried out a meta-analysis of five peer-reviewed studies that screened 50 576 people in the general population (75 534 alleles) for expanded alleles of the *FMR1* gene.[13 14 31–33] In those studies, a total of 186 premutation alleles were detected.

Allele size distributions were first compared for homogeneity across the five population-based studies to determine whether pooling was acceptable. Next, the aggregate allele size distribution from population-based studies was compared with that for patients with FXTAS, both with and without a family history. Size distributions were grouped by ordinal category to reflect reporting in the literature. All comparisons of allele-group frequencies used rank-based tests (Wilcoxon's rank sum test) to account for the ordered categories without ignoring unequal widths of other categories and to enhance power for testing for a shift up or down in allele distribution. The distributions of numbers of repeats were compared across pairs of groups using Wilcoxon's rank sum test. All tests were two-sided, level 0.05, and all analyses were carried out using SAS.

RESULTS

Prevalence of the premutation allele among movement disorder populations

Fourteen screening studies, comprising a total of 3265 adults with movement disorders, were published between 2003 and 2005.[6 16–28] Among these studies, the prevalence of the premutation varied from 0% to 5%, depending on the diagnostic category. Table 1 presents a compilation of the published results. Of the 3265 patients screened, 22 premutation alleles were identified, ranging in size from 61 to 135 CGG repeats, establishing the overall prevalence of the *FMR1* premutation among the screened patients with movement disorders as 0.8% (18/2330) in men and 0.5% (4/935) in women. The prevalence in men is significantly greater than that in the general population ($p<0.001$), due in large part to increases within the ataxia and MSA-C categories; however, the figure for women is not significantly different from the prevalence of the premutation allele in the general population (table 1).

When broken down into separate diagnostic categories (table 1), the prevalence of premutation alleles among patients with a diagnosis of cerebellar ataxia was 1.5% for men (16/1049; odds ratio (OR) 12.4, 95% confidence interval (CI) 1.6 to 93.5), which is 13 times greater than the prevalence in the general population; the prevalence was 0.2% (1/549) for women, which is similar to the general population. In contrast, premutation alleles were absent among screened patients who met the criteria for Parkinson's disease (0/656) and essential tremor (0/348).

Five carriers of premutation alleles (three women and two men) who met the strict criteria for probable MSA[29] were identified among 663 patients with a prior diagnosis of MSA (280 MSA-C and 341 MSA-P).[16 19 22 26] Although these aggregate numbers of expanded alleles among the MSA population (all diagnostic categories) were not significantly different from the prevalences of the premutation in either the general male or female population (men: OR 4.4, 95% CI 0.4 to 48.6; women: OR 3.1, 95% CI 0.3 to 29.5), the numbers

Table 1 Distribution and *FMR1* testing results for patients referred to movement disorder clinics

	Premutation allele/sample size		
Clinical diagnosis	Men	Women	Study
PD	0/605	0/0	Ref nos 22, 23, 27
Atypical PD	0/51	0/11	Ref nos 22, 23
Essential tremor	0/270	0/78	Ref nos 17, 22, 27
MSA	2/366	3/297	Ref nos 16, 19, 22, 26
MSA-P*	1/341		
MSA-C*	4/280 (3/76)†		
Cerebellar ataxia‡	16/1049§	1/549	Ref nos 6, 16, 18, 20–22, 24, 25, 28
Total	18/2330	4/935	

MSA, multiple system atrophy; MSA-C, multiple system atrophy-cerebellar; MSA-P, multiple system atrophy-parkinsonism, PD, Parkinson's disease.
*Includes possible, probable and definite diagnostic categories [29]; sex distributions for MSA-C and MSA-P were not provided in all articles.
†§Significantly higher (probable MSA-C, $p<0.004$; cerebellar ataxia A, $p<0.001$) than the prevalence of the premutation allele in the general population.
‡Includes 19 cases of olivopontocerebellar atrophy.
Parentheses indicate the subgroup of probable MSA-C.[26]

do reach significance when we consider only the probable MSA-C subgroup (3/76 cases [26];and 1/55 cases[16])—that is, excluding definite MSA-C—which requires pathological confirmation of oligoglial cytoplasmic inclusions. Indeed, even assuming the higher background frequency of about 1 in 250 for women,[13] 3/76 (one woman, two men with reported probable MSA-C) is highly significant ($p<0.004$). Moreover, the clinical significance of these numbers becomes even greater if we take into consideration the larger allele sizes for the two men (111, 71 CGG repeats), where the population frequencies drop below 1 of 1000.

Clinical and radiological presentation of premutation carriers identified through movement disorder population screening and family-based studies

In all but three cases (49, 48 and 10 years),[20 21 26] the onset of symptoms occurred after the age of 50 years. All premutation carriers identified through the movement disorders screening studies presented with cerebellar ataxia. The movement disorder diagnoses included 17 cases of cerebellar ataxia (including one case of olivopontocerebellar atrophy), four cases of MSA-C and one case of MSA-P. As previously mentioned, no patient met the criteria for Parkinson's disease or essential tremor. Table 2 shows the data on clinical and brain magnetic resonance imaging.

Grey zone alleles

Nine studies reported grey zone alleles (defined variously as 45–54 or 41–54 CGG repeats among the various studies) within their screened populations.[6 16 17 19 20 23 24 26 27] A total of 67 grey zone alleles (38/1658 men and 29/583 women) were reported among these screening studies, resulting in prevalence values of 2.3% for men and 5% for women; these values are not significantly different from the prevalence of this allele in the general population.[15 34]

Comparison of premutation allele distributions in the general population and in FXTAS patient groups

For premutation carriers identified through populations with movement disorders (without a family history), allele sizes were reported in 22 patients (mean 92 CGG repeats; range 61–135 repeats; SD 16 repeats). In all, 87% (19/22) of the alleles were larger than 70 repeats and 77% were larger than 80 repeats (table 3).

For premutation carriers identified through fragile X family studies, allele size was available in 46 patients (mean, 83 CGG repeats; range, 55–107 repeats; SD, 12 repeats).

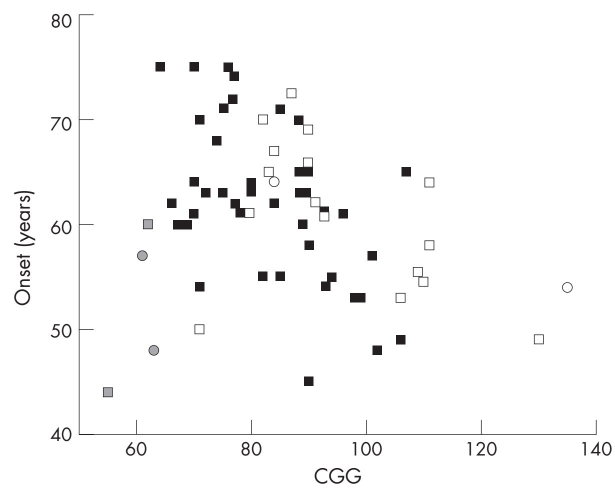

Figure 1 Distribution of CGG repeats and ages of onset for the premutation patients described in the current study. Men (squares, n = 18*) and women (circles, n = 4) without a family history of fragile X syndrome are indicated by open symbols. Men ascertained through family studies (n = 46) are indicated by black squares. The patients with atypical clinical presentation are identified by grey symbols. *Two of the cases are not represented on the graph; in one case, the age of onset was during childhood [20]; in the second case, the age of onset was not reported.[25]

For premutation carriers identified through population-based studies, allele sizes were available in 183 patients and they were reported by ordinal categories (table 3).

The two FXTAS groups (with and without a fragile X family history) did not differ significantly in their distribution of allele size ($p = 0.18$), with both groups having most alleles >80 repeats (table 3). We therefore elected to pool those two groups for the purpose of comparison with the allele size distribution in studies conducted in the general population. We verified that it was legitimate to pool the three population-based studies on men[14 32 33] and the two population-based studies on women.[13 31] Overall, we found that the population-based studies showed no differences in their allele distributions: Dombrowski versus Rife, $p = 0.47$; Dombrowski versus Tzeng, $p = 0.59$; Rife versus Tzeng, $p = 0.39$ (table 3). The distribution of allele sizes for patients with FXTAS was significantly skewed towards larger alleles relative to the distribution of alleles in the general population ($p<0.001$; table 3; fig 2). For general population studies,

Table 2 Distribution of clinical and radiological symptoms in the patients with fragile X-associated tremor/ataxia syndrome identified through neurology clinics and family-based studies

	Movement disorder screening studies, n = 22*	Family-based studies, n = 64[1–3]
Age of onset (SD)†	60 (13) years	63 (8) years
Mean number of CGG repeats‡	92 (16)	84 (16), n = 46§
Gait ataxia	100%	95%
Cognitive decline	50%	20% (6/29)¶
Intention tremor	55%	88%
Incontinence/ dysautonomic features	42%	34%
Rigidity/bradykinesia	29%	50%
Pyramidal symptoms	9%	—
Sensory loss	21%	28%
MRI presentation		
MCP signal intensity	76% (13/17)	64% (21/33)**
Atrophy	85%	84%

MCP, middle cerebellar peduncle; MRI, magnetic resonance imaging.
*Clinical descriptions were available for 21 of 22 patients identified through genetic screens.[6 16 18 21 24 26]
†Figure 1 shows the distribution of age of onset.
‡Table 3 shows the distribution of allele sizes.
§Exact allele size was obtained for 46 of 64 cases.
¶29 patients received cognitive testing and 6 of 29 had a full-scale IQ <85, which is reflective of significant IQ decline or dementia.
**33/46 have MRI results.

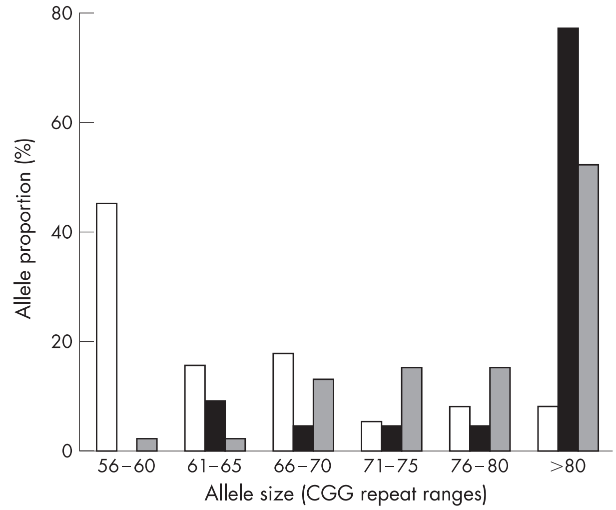

Figure 2 Comparison of the distribution of premutation alleles among carriers with neurological involvement with the corresponding distribution of premutation alleles from five population-based studies. In premutation carriers with fragile X-associated tremor/ataxia syndrome, allele sizes are significantly shifted towards large expansions ($p<0.001$). General population (n = 186; white bars); ascertainment through movement disorder screening studies (n = 22; black bars); and ascertainment through studies of known fragile X families (n = 46; grey bars).

approximately 80% of premutation alleles were <70 repeats, regardless of sex and ethnicity.[13 14 31–33]

DISCUSSION

The current meta-analysis has shown that of the adult-onset movement disorders that have a clinical overlap with FXTAS, genetic screens (3265 adults) for premutation expansions of the *FMR1* gene have had the greatest overall yield among men with an initial clinical diagnosis of late-onset cerebellar ataxia (1.5%). However, this is likely to be an underestimate of the true prevalence of FXTAS as the cause of late-onset ataxia in men. Only approximately 4% of people with FXTAS diagnosed in family studies are evaluated by movement disorder neurologists, whereas the rest are seen by general neurologists or primary care physicians.[35] The primary care physicians are less likely to order genetic testing for ataxia (eg, only 1/70 medical charts reviewed by MAL indicated spinocerebellar ataxia genetic testing). Thus, patients ascertained through the movement disorder screening studies represent only a subgroup of patients with FXTAS having cerebellar ataxia as a prominent component of their clinical presentation. Another reason that the 1.5% figure may be an underestimate of the number of men with late-onset ataxia likely to have FXTAS is that the overall mean (SD) age of onset in the cases screened was 53.4 (5.8) years (based on data available in 7/14 studies, range: 42–67 years[16–18 21–23 27]). Therefore, approximately 22% of the patients in these cohorts had an onset of symptoms before the age of 50 years, representing a dilution effect of younger cases that are unlikely to have FXTAS.

However, it is also possible that the screening of populations with movement disorders that are not ascertained through known fragile X syndrome probands may identify cases with earlier onset of neurological symptoms, such as the three cases (49, 48 and 10 years) described by Macpherson *et al*,[20] Kamm *et al*,[26] and Seixas *et al*.[21] Two of

Table 3 Premutation alleles size distribution in the general population and in fragile X-associated tremor/ataxia syndrome patients with and without a family history of fragile X

	Premutation alleles in population-based studies						FXTAS	
	Toledano *et al*[31]	Dombrowskl *et al*[14]	Tzeng *et al*[33]	Rife *et al*[32]	Rousseau *et al*[13]	Total	Movement disorder screening studies	Family based
Population size	14334	10572	10046	5000	10624	50576	3265	NA
No of alleles	28668	10572	10046	5000	21248	75534	4200	NA
Sex	F	M	M	M	F	M/F	M/F	M
No of premutation alleles								
55–60 repeats	62	6	2	3	11	84	0	1
61–65 repeats	15	3	1	0	10	29	2	1
66–70 repeats	25	1	1	0	6	33	1	6
71–75 repeats	4	0	0	0	6	10	1	7
76–80 repeats	9	1	1	1	3	15	1	7
81–200 repeats*	9	2	1	0	3	15	17	24
Total	124	13	6	4	39	186	22	46

F, female; FXTAS, fragile X-associated tremor/ataxia syndrome; M, male; NA, not applicable.
*Premutation alleles >80 repeats are rare in the general population in comparison with smaller alleles. In patients with FXTAS, most alleles are >80 repeats.

those cases[20 26] could represent coincidental findings: their clinical and radiological presentations were atypical and alleles were small (63 and 66 CGG repeats, respectively). However, the third case reported by Seixas *et al*[21] (onset at 49 years) met the criteria for ''definite FXTAS'',[2] with a very large allele of 130 CGG repeats.

Clinically, there were strong similarities between patients with FXTAS recruited with and without a family history of fragile X syndrome (table 2). The age of onset and distribution of symptoms were identical except for intention tremor, which was more common in the patients ascertained through the fragile X syndrome probands. In the family-based studies,[1–3 7 8] the biased allele size among patients with FXTAS has been attributed to the ascertainment methods—that is, all patients reported were second-degree relatives of a child affected with fragile X syndrome, requiring expansion or transmission of a full mutation allele to the fragile X syndrome proband in two generations. In contrast, for the patients identified through populations with movement disorders, the ascertainment bias of family-based studies is avoided. Nevertheless, the allele size distribution remains shifted towards larger premutation expansions, with 19/22 alleles exceeding 70 CGG repeats.

The allele distributions for both family-based and movement disorder-based modes of ascertainment were significantly different from the observed distribution in the general population, where approximately 80% of the premutation alleles are <70 repeats. Therefore, larger CGG repeats would represent an increased risk factor for the development of FXTAS; this suggestion is consistent with recent evidence that both the age of onset and the age of death seem to depend on the CGG repeat size[10](Tassone *et al*, unpublished results), as does the degree of brain atrophy.[9]

We cannot say at this point whether there is a strict lower limit for the size of the CGG repeat required for clinical involvement in FXTAS; however, clinical presentation is often atypical in those patients with smaller allele sizes, and the findings may be coincidental: the patient with 66 CGG repeats, reported by Macpherson *et al*,[20] presented with an unusual, early-onset ataxia at 10 years of age, with no abnormal MCP finding on magnetic resonance imaging. The two women carriers reported by Kamm *et al*,[26] with 63 and 61 repeats, both had atypical MRI findings comprising the ''hot cross bun'' sign in the pontine fibres and increased signal intensity (T2-weighted image) in the dorsolateral putamen. These MRI findings are typical of MSA and have not been reported in patients with FXTAS. These atypical patients, and atypical cases from the family-based studies, are identified in fig 1 with grey symbols. Finally, in two postmortem studies of carriers with small CGG-repeat expansions in the 60s, there was only minimal neuropathology, with infrequent-to-rare inclusions in three cases with CGG repeats in the 60s studied, and no evident cerebral or cerebellar white matter disease (Greco *et al*, unpublished results).[10]

The apparent bias towards larger premutation alleles in the screened population with movement disorders suggests that the true prevalence of FXTAS in the general population may be lower than the initial estimates,[2] which were based on the frequency of premutation alleles in the general population. If we restrict the range of clinical involvement of FXTAS to those patients with premutation alleles that ⩾70 CGG repeats (approximately 22% of the total number of screened premutation alleles), with a penetrance of approximately 40% for male carriers >50 years, then the overall penetrance for men <50 years could be as low as 1 in about 9000, subject to substantial uncertainty for the overall prevalence of premutation alleles in the general population. However, inclusion of slightly smaller alleles, in the 66–70-repeat range, would double the predicted penetrance, to about 1 in 4500. This uncertainty notwithstanding, the lifetime risk would be somewhat higher (approximately 1/6000 to 1/3000), because of the increasing penetrance with age.[2] Again, this figure would not take into account the prospect of milder phenotypic involvement in carriers of even smaller alleles. This uncertainty in the prevalence of clinical involvement among premutation carriers underscores the need for additional screening studies on a larger scale and penetrance studies for smaller premutation alleles. Recommended screening criteria include onset of otherwise unspecified cerebellar ataxia or probable MSA-C in an individual >50 years of age; or onset of non-resting tremor of unknown cause in an individual <50 years of age, who has one or more of the following additional features: MCP sign on T2/fluid-attenuated inversion recovery imaging, a positive family history of infertility/premature menopause, or a family history of the *FMR1* mutation.

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